Thermo SCIENTIFIC

PRODUCT INFORMATION

ThermoScientific
DreamTaq Green PCR Master Mix (2X)

#K1081 200 rxns

Lot: __ Expiry Date: __

Store at -20°C

Ordering Information

Component	#K1081 200 rxns of 50 µL	#K1082 1000 rxns of 50 µL
DreamTaq Green PCR Master Mix (2X)	4 × 1.25 mL	20 × 1.25 mL
Water, nuclease-free	4 × 1.25 mL	20 × 1.25 mL

www.thermoscientific.com/onebio

Description

Thermo Scientific DreamTaq Green PCR Master Mix (2X) is a ready-to-use solution containing DreamTaq™ DNA polymerase, optimized DreamTaq Green buffer, $MgCl_2$ and dNTPs. The master mix is supplemented with two tracking dyes and a density reagent that allows for direct loading of the PCR product on a gel. The dyes in the master mix do not interfere with PCR performance and are compatible with downstream applications such as DNA sequencing, ligation and restriction digestion.
The master mix retains all features of DreamTaq DNA polymerase. It is capable for robust amplification up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
For applications that require PCR product analysis by absorbance or fluorescence excitation, we recommend using the colorless DreamTaq PCR Master Mix (2X), #K1071.

Rev.5

Applications

- Routine PCR amplification of DNA fragments up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
- RT-PCR.
- Genotyping.
- Generation of PCR products for TA cloning.

DreamTaq Green PCR Master Mix (2X) composition

DreamTaq DNA polymerase is supplied in 2X DreamTaq Green buffer, dATP, dCTP, dGTP and dTTP, 0.4 mM each, and 4 mM $MgCl_2$. DreamTaq Green buffer is a proprietary formulation optimized for robust performance in PCR. It contains a density reagent and two dyes for monitoring electrophoresis progress:
the blue dye migrates with 3-5 kb DNA fragments in a 1% agarose gel and the yellow dye migrates faster than 10 bp DNA fragments in 1% agarose gel. The dyes have absorption peaks at 424 nm and 615 nm.

PROTOCOL

1. Gently vortex and briefly centrifuge DreamTaq Green PCR Master Mix (2X) after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 µL reaction:

DreamTaq Green PCR Master Mix (2X)	25 µL
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA	10 pg - 1 µg
Water, nuclease-free	to 50 µL
Total volume	50 µL

3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Perform PCR using the recommended thermal cycling conditions outlined below:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	1-3 min	1
Denaturation	95	30 s	25-40
Annealing	Tm-5	30 s	
Automated fluorescent extension	72	1 min/kb	
Final extension	72	5-15 min	1

6. Load 5-15 µL of PCR mixture directly on a gel.

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform “no template control” (NTC) reactions to check for contamination.

GUIDELINES FOR PRIMER DESIGN

Use the Thermo Scientific REviewer primer design software at www.thermoscientific.com/reviewer or follow the general recommendations for PCR primer design as outlined below:

- PCR primers are generally 15-30 nucleotides long.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3’-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3’-end.
- Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementary between primers and template DNA.
- When designing degenerate primers, place at least 3 conservated nucleotides at the 3’-end.
- When introducing restriction enzyme sites into primers, refer to the table “Cleavage efficiency close to the termini of PCR fragments” located on www.thermoscientific.com/onebio to determine the number of extra bases required for efficient cleavage.

Estimation of primer melting temperature

For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:

$$Tm = 4 (G + C) + 2 (A + T),$$

where G, C, A, T represent the number of respective nucleotides in the primer.
If the primer contains more than 25 nucleotides we recommend using specialized computer programs e.g., REviewer™ (www.thermoscientific.com/reviewer) to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amounts of template DNA for a 50 µL reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Higher amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.
All routine DNA purification methods can be used to prepare the template e.g., Thermo Scientific GeneJET Genomic DNA Purification Kit (#K0721) or GeneJET™ Plasmid Miniprep Kit (#K0502). Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol usually removes trace contaminants from DNA samples.

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products.
For degenerate primers and primers used for long PCR we recommend higher primer concentrations in the range of 0.3-1 µM.

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CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation

It is essential to completely denature the template DNA at the beginning of PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95°C is sufficient.

Denaturation

A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min.

Primer annealing

The annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments.

Extension

The optimal extension temperature for DreamTaq DNA polymerase is 70-75°C. The recommended extension step is 1 min/kb at 72° for PCR products up to 2 kb. For larger products, the extension time should be prolonged by 1 min/kb. For amplification of longer templates (>6 kb) a reduction of the extension temperature to 68°C is required to avoid enzyme inactivation during prolonged extension times.

Number of cycles

If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.

Final extension

After the last cycle, it is recommended to incubate the PCR mixture at 72°C for an additional 5-15 min to fill-in any possible incomplete reaction products. If the PCR product has to be cloned into a TA vector, e.g. using Thermo Scientific InsTAclone PCR Cloning Kit (#K1213), the final extension step may be prolonged to 30 min to ensure the highest efficiency of 3'-dA tailing of the PCR product. If the PCR product has to be used for cloning using Thermo Scientific CloneJET PCR Cloning Kit (#K1231), the final extension step can be omitted.

Troubleshooting

For troubleshooting please visit
www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 25 µL of DreamTaq Green PCR Master Mix (2X) with 1 µg of pUC19 DNA in 50 µL of reaction mixture for 4 hours at 37°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA/HindIII fragments was observed after incubation of 25 µL of DreamTaq Green PCR Master Mix (2X) with 1 µg of digested DNA in 50 µL of reaction mixture for 4 hours at 37°C.

Ribonuclease Assay

Less than 0.5% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25 µL DreamTaq Green PCR Master Mix (2X) with 1 µg of [^{3}H]-RNA in 50 µL of reaction mixture for 4 hours at 37°C.

Functional Assay

DreamTaq Green PCR Master Mix (2X) was tested for amplification of 956 bp single copy gene from human genomic DNA and for amplification of 20 kb lambda DNA fragment.

Quality authorized by:  Jurgita Zilinskiene

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